



Research Article

Ecotoxicological Aspects of Pharmaceuticals on Aquatic Environment

Prabal Giri ^{1*} and Churala Pal ²¹ Department of Chemistry, Guskara Mahavidyalaya, Burdwan, West Bengal, India² Department of Chemistry, Basanti Devi College, Kolkata, West Bengal, India**Abstract**

Research on pharmaceuticals is becoming important today to the chemists as well as to the biologists considering the complex molecular functionalities, diverse physicochemical properties and bioactivities. Specific biological activities are the main reason for their use and development. Biodegradation modifies the chemical structure of their active molecules resulting in a change in their physicochemical and pharmaceutical properties. Chronic ecotoxicity data as well as information on the current distribution levels in different environmental compartments continue to be sparse and are focused on those therapeutic classes. Nevertheless, they indicate the negative impact that these chemical contaminants may have on living organisms, ecosystems and ultimately, public health. This paper describes the different contamination sources as well as fate and both acute and chronic effects on non-target organisms.

Keywords: Supramolecular Chemistry; Crystal Engineering; Co-crystals; Pharmaceutical Co-crystals**Reviewer:** Bina Kumari, Annamalai University, India**Received:** September 17, 2014; **Accepted:** October 17, 2014; **Published:** November 5, 2014

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Copyright: 2014 Giri P *et al.* This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.***Correspondence to:** Prabal Giri, Department of Chemistry, Guskara Mahavidyalaya, Burdwan, West Bengal, India. Email: prabal25@gmail.com

Introduction

Pharmaceutically active compounds are complex molecules with different functionalities, physicochemical and biological properties. They are developed and used because of their more or less specific biological activity and are most notably characterised by their ionic nature. Their molecular weights range typically from 300 to 1000. Under environmental conditions molecules can be neutral, cationic, anionic, or zwitterionic. They also often have basic or acidic functionalities. Pharmaceuticals can be classified according to their effects, but also “crosswise” according to their chemical structure. Normally, pharmaceuticals and disinfectants are classified according to their therapeutic purpose (e.g. antibiotics, analgesics, antineoplastics, anti-inflammatory substances, antibiotics, antihistaminic agents, contrast media, etc.). Powerful hyphenated chromatographic-detection techniques enabling detection upto the ng/ L allowed researchers to quantify a large number of medicines components (i.e. drugs and excipients) in the environment, thus compelling the scientific community to consider this contamination type as a potential issue meriting concern [1-3]. In fact, tons of them are produced annually worldwide to be consumed by humans or animals [4,5]. They are conceived primarily to have particular physiological modes of action and frequently to resist to inactivation before exerting their intended therapeutic effect. However, these same properties are paradoxically responsible either for bioaccumulation and toxic effects in aquatic and terrestrial ecosystems [6,7]. In a different way from some conventional pollutants (such as pesticides, detergents, fuels, among others), medicines are continuously delivered at low levels which might give rise to toxicity even without high persistence rates [8-10]. Wide dissemination at low concentrations mainly in the aquatic environment is evident today. Such concentrations have been detected in aquatic compartments such as influents [11-13] and effluents [14-16] from sewage treatment plants (STPs), surface waters (rivers, lakes, streams, estuaries, among others) [17-21], seawater [22], groundwater [23-25] and drinking water [26-29]. The scientific community is in broad agreement with the possibility that adverse effects may arise from the presence of pharmaceuticals not only for human health but also for aquatic organisms. Several, almost negligible effects have been shown to occur from continuous exposure during the life cycle of aquatic vertebrates and invertebrates to sub-therapeutic drug concentrations [30,31]. These effects slowly accumulate to manifest themselves into a final irreversible condition which is frequently only noticed several generations’ later, affecting sustainability of aquatic organisms’ populations [32].

This paper presents an updated survey of the acquired knowledge regarding the sources, spreading conditions, occurrence and induced toxic effects on non-target organisms by drugs in the environment.

Sources of environmental contamination

The most obvious pathway for environmental contamination of medicines is via the unaltered excretion in urine and faeces although other anthropogenic mechanisms should be assumed, namely:

(A) Metabolism post-consumption; since many drugs are metabolised as the organism attempts to convert hydrophobic compounds into more easily excreted polar residues. Their bioconversion

into one or more metabolites can occur throughout Phase I and Phase II reactions [33] as shown in Fig. 1.

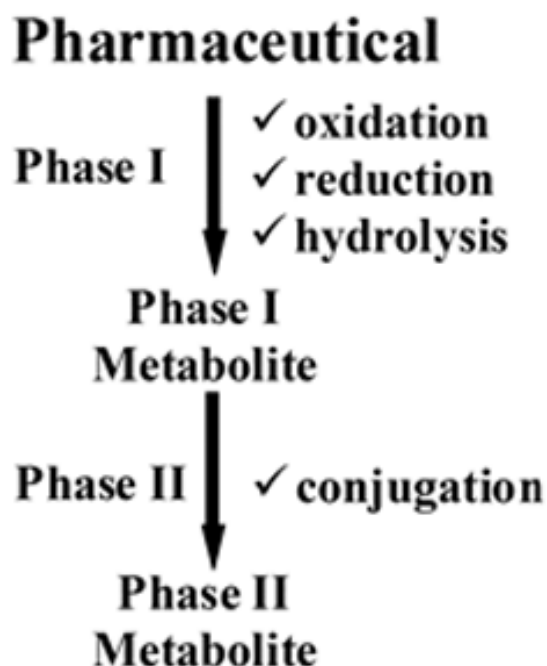


Figure 1 Schematic representation of pharmaceutical biotransformation

(B) Diagnostic compounds; such as X-ray contrast media are directly discharged in their native forms. (C) Household Disposal; either topic formulations or unused medicines (out-of-date or unwanted) are discarded through the sink/toilet or via waste collection [34,35], before being taken to landfill sites where they appear as terrestrial ecosystem contaminants. Alternatively, they may possibly leak into surrounding water compartments [36,37].

(D) Impacts due to anthropogenic activities; as, for instance, Sewage Treatment Plant (STP) sludge, which can carry non-suspected drugs and is frequently used as a fertilizer on agricultural land [38,39]; veterinary medicines, which are also excreted in urine and faeces by animals before being spread onto land via manure application as fertilisers. Apart from the potential for direct soil contamination, there is also the risk of run-off with heavy rain, thus potentially contaminating both the surrounding surface and groundwater [40-42]. Other example of an anthropogenic activity is aquaculture, whose pharmaceuticals employed, as well as their metabolites and degradation products, are directly discharged into surface waters [43,44]. Another important source of environmental contamination by pharmaceuticals is the effluents of pharmaceutical production facilities [45-47].

At a higher level, existing geographical information on environmental contamination sources is sparse and limited. Countries and regions worldwide differ concerning the prevalence of diseases, waste treatment processes, cultural habits or economic constraints related to the pharmaceutical market. Nevertheless, it seems that urban regions are major sources of contamination due to the proximity of hospitals and STP facilities. Additionally, the contribution of rural regions where

agriculture, animal husbandry and aquaculture represent important ways of life should be considered as important.

Environmental fate

The fate and behaviour of medicines in the environment still requires further elucidation. As previously stated, drugs (used in human and/or in veterinary medicine) and their metabolites are spread into the environment in different ways, namely through STP effluents, heavy rain on agricultural land provokes (surface) water run-off, and occasionally, through untreated sewage (domestic wastes and flooding, among others) as shown in Fig. 2.

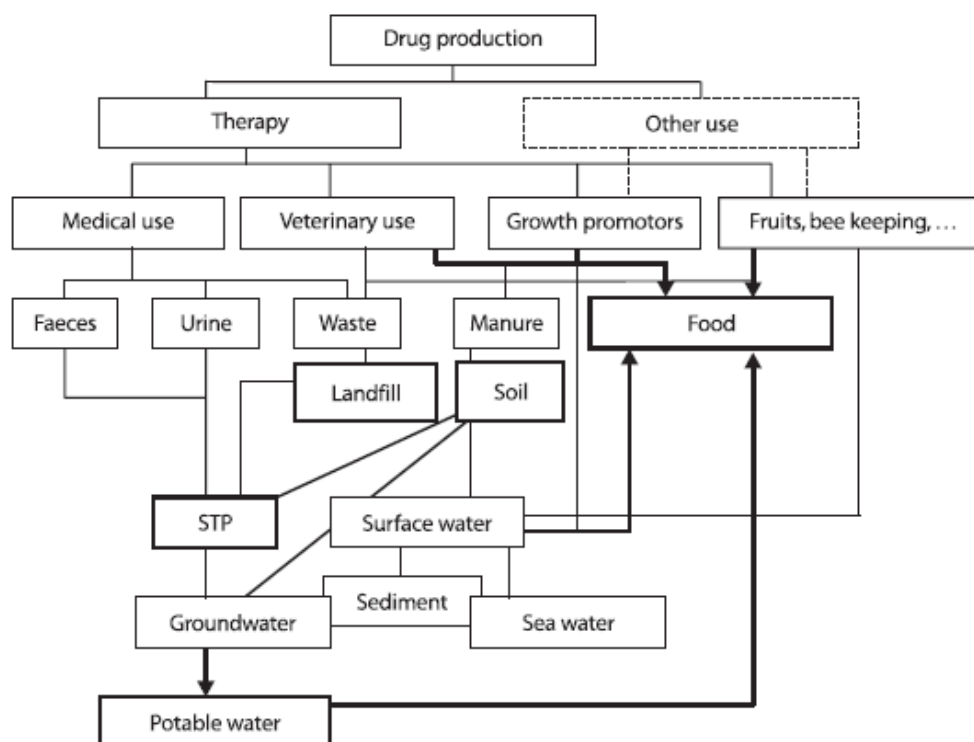


Figure 2 Sources, distribution and sinks of pharmaceuticals (adapted from Ref. 1).

Some of them do reach surface waters (rivers, lakes and estuaries, among others) and eventually ground waters after resisting the intended biological degradation. However, in surface waters they may be degraded through different processes such as photolysis whose efficiency depends on factors such as intensity of solar irradiation, latitude, season of the year and presence of photosensitizers (e.g. nitrates, humic acids) [48,49].

In the case of drugs that have low volatility and high polarity distribution is mainly made by aqueous transport or even via food chain dispersion [50]. Usually, wastewaters are conducted to STPs, which play a key role in the entrance of pharmaceuticals in the environment. However, in some

regions or even countries these kinds of facilities may not exist and the environmental problem is still worse. The evaluation of removal efficiency in STPs (by comparing influent and effluent contents) has been studied in detail, showing removal rates that can differ by up to 99% [51-53]. Depending both on the particular technology resorted to and the active substance properties they may undergo: (i) degradation (mineralization) to low molecular weight compounds (e.g. CO₂ and water); (ii) entrapment by suspended solids; (iii) discharge of the parent compound through chemical cleavage of the respective conjugate forms and (iv) conversion to a more hydrophilic, persistent form which will short-circuit the treatment process [54,55]. Thus, in hospitals use of specific antibiotics, antineoplastic or diagnostic agents subsequently requires a sewage treatment process more embracing and directed to these kind of drugs, which are only used in hospitals [56], and that must be different to the more specific procedure adopted at STPs receiving industrial discharges from drug manufactures [57]. In both, the form and extension of the final contamination risk will also depend on geographical location of the STP facility. Low adsorption coefficients that make active substances remain in the aqueous phase, favour their mobility through the STP and into nearby surface waters [51]. Adsorption to suspended solids depending on both hydrophobic and electrostatic interactions established between each will follow the same destiny [9,39]. On the other hand, hydrophobic metabolites will be held on STP sludge, provoking terrestrial contamination, thus affecting microorganisms and invertebrates. Aerobic/anaerobic bio-conversion occurring either during sewage sludge digestion or during activated sludge treatment seems to be the most efficient process to eliminate chemical contaminants from the aquatic environment. Usually, the best biodegradation results are obtained when activated sludge treatment is conducted through an increase in hydraulic retention time and the use of mature sludge [8]. However, one should be aware of the fact that if a particular pharmaceutical is not detected in a STP effluent, this does not imply that it has been fully removed. On some occasions, it may have been degraded and give rise to unsuspecting metabolites that will subsequently contaminate surface waters [58]. Notwithstanding that some drugs and their metabolites show a stable nature, nowadays is still difficult to establish a complete contamination pattern in final receiving surface waters, due to the water dilution, the treatment and discharging processes [52].

Ecotoxicology

Continuous consumption of drugs even at sub-therapeutic concentrations represents a potential threat to public health although one should bear in mind that it is still impossible to evaluate the effects of exposure on human health [58,59]. In turn, many non-target organisms (which possess human- and animal-alike metabolic pathways, similar receptors or biomolecules) are therefore inadvertently exposed to active substances released into the environment [8,33]. A comprehensive manner to evaluate the toxicity effects on non-target organisms must include the development of specific tests embracing either acute effects (where mortality rates are often registered) or chronic effects (by means of exposure to different concentrations of a chemical compound over a prolonged period of time). In the latter, effects are measured through specific parameters such as growth index or reproduction rates [50]. Unfortunately, studies on acute effects in organisms belonging to different trophic levels (i.e. algae, zooplankton and other invertebrates and fish) predominate relatively to chronic ones (Fig. 3).

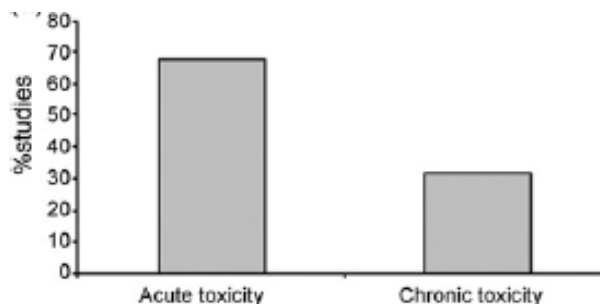


Figure 3 Plot of acute versus chronic ecotoxicological data

Acute toxicity data is only valuable when accidental discharge of the drugs occurs, since the environmental concentrations usually reported for these compounds are low, typically in a factor of one thousand. Bioaccumulation and chronic toxicity tests are scarce [8,33] probably due to the complex experimental work involved. However, recent development of sensitive methods for identification and quantification of drugs enabled to devise their distribution patterns in several environmental samples, thus highlighting the more relevant therapeutic classes in terms of environmental contamination as presented by Fig. 4. These data is useful to set out the most appropriate active substances to be used in ecotoxicity tests. According to data present in literature, scientific community has mainly concerned their attention on therapeutic classes such as, non-steroidal anti-inflammatory drugs, blood lipid lowering agents, antibiotics and sex hormones. By those reasons, this review will focus in the drugs belonging to those therapeutic classes. Within this context, some of the acute and chronic toxicity effects caused by drugs belonging to different therapeutic classes and mixtures of them in non-targets organisms deserve further analysis and are discussed in the following section. For a critical analysis of the ecotoxicological data present in the literature relatively to different drugs, we decide to group them according to their main pharmacological activity. Therefore, toxicity data will be related to the environmental concentrations found by several authors, to establish the severity of the situation.

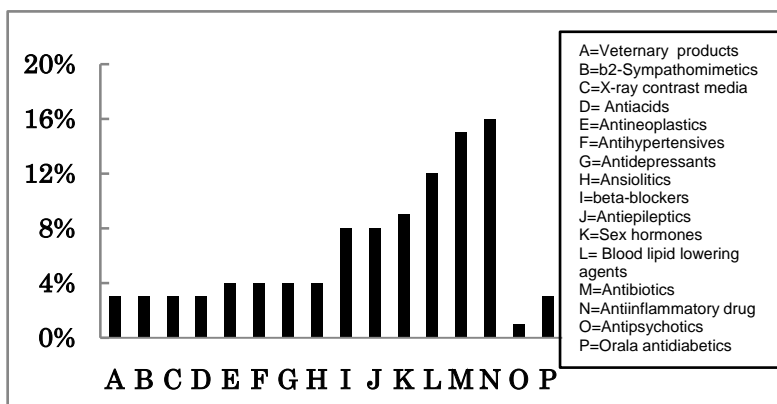


Figure 4 Graphical representation showing therapeutic classes detected in environment expressed in terms of relative percentage

Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAID) are weak acids acting by reversible or irreversible inhibition of one or both isoforms of the cyclooxygenase enzymes, COX-1 and COX-2, involved in the synthesis of different prostaglandins from arachidonic acid [61]. A cyclooxygenase enzyme similar to human COX-2 has been found in fish thereby making them a potential target for aquatic contamination [62]. Prostaglandins also play an important role in the synthesis of bird eggshells and from inhibiting its synthesis, shell thinning has been observed [63]. Among the NSAID, diclofenac showed the most acute toxic nature with effects being observed at concentrations below 100mg/ L [64]. Chronic toxicity trials performed on rainbow trout (*Oncorhynchus mykiss*) evidenced cytological changes in the liver, kidneys and gills after 28 days of exposure to just 1g/ L of diclofenac. For a concentration of 5 g/ L renal lesions were evident as well as drug bioaccumulation in the liver, kidneys, gills and muscle [65,66]. Brown trout (*Salmo trutta f. fario*) showed similar cytological damage and a reduction of haematocrit values after 21 days of exposure to 0.5 g/ L of this active substance [67]. Schmitt-Jansen *et al.* [68] evaluated both diclofenac phytotoxicity and its photochemical products on the unicellular chlorophyte *Scenedesmus vacuolatus*. Inhibition of algal reproduction by the parent compound only occurred at a concentration of 23 mgL⁻¹, hence indicating no specific toxicity. However, the threat significantly increased when metabolites were produced from 53 h of exposure to daylight. Diclofenac also inhibited the growth of marine phytoplankton *Dunaliella tertiolecta* for concentrations of 25mg/ L and above [69]. For this organism, 96 h EC₅₀ of 185.69mg/ L was found [69]. Diclofenac was detected in STP effluents at maximum concentrations of 2.4 [13] and 1.42 g/ L [70] in Switzerland and Belgium respectively which highlighted that the effects cited are of sufficient magnitude to suspect chronic toxicity in aquatic organisms. Diclofenac has also been found in rivers [20,71], groundwater [24], hospital effluents [45,72] and drinking water but at concentrations in the order of ng/ L.

Ibuprofen is another NSAID with documented chronic toxicity. Female Japanese medaka (the Japanese killifish, *Oryzias latipes*) exposed to different concentrations of the drug over six weeks, showed a sharp rise in liver weight together with enhanced egg production, yet with a reduction in the number of weekly spawning events [73]. The ecotoxicity of naproxen and its photoderivative products have also been envisaged. Acute toxicity tests performed on the rotifer *Brachionus calyciflorus*, the water flea *Ceriodaphnia dubia* and the fairy shrimp *Thamnocephalus platyurus*, showed that naproxen had LC₅₀ 4 and EC₅₀ 5 values within the 1-100 mg/ L range, with the photolysis products being significantly more toxic [74]. Highly chronic toxic properties were equally noticed with algae being the less sensitive organisms. The highly prescribed paracetamol (or acetaminophen) is a weak inhibitor of the cyclooxygenase enzyme, whose side effects are mainly associated with the formation of hepatotoxic metabolites, such as N-acetyl-p-benzoquinone imine (NAPQI) when the levels of liver glutathione are low. Tests were carried out on algae, water fleas, fish embryos, luminescent bacteria and ciliates. The most sensitive species was shown to be *D. magna* for which EC₅₀ values of 30.1 [75] or 50 mg/ L [76] were reported.

Antibiotics

Antibiotics come within a therapeutic class where human health preservation and environmental disturbance are closely related. The major concern is associated with the development of resistance mechanisms by bacteria which can subsequently compromise public health by means of treatment effectiveness [77]. According to Jones et al. [78], antibiotics could be classified as extremely toxic to microorganisms (EC_{50} below 0.1mg/ L) and very toxic to algae (EC_{50} between 0.1 and 1mg/ L). Most antibiotics used in veterinary medicine are aimed at preventing and treating diseases in livestock production or aquaculture. Even considering their use at sub-therapeutically concentrations, many studies suggest the development of bacterial resistance and further potential appearance of cross-resistance between different classes of antibiotics shared with humans [78,79]. Antibiotics used in livestock production are excreted in the urine and faeces of animals and often appear in manure. From here they can cause some problems in terrestrial ecosystems such as adverse effects on nitrifying bacteria [9] or growth inhibition of crop plants and weeds by bioaccumulation [80]. Bacterial cultures from sewage bioreactors receiving waters from a STP were tested for resistance against six antibiotics, showing that all were resistant to at least two of the antibiotics, whilst bacteria isolated from receiving waters were only resistant to erythromycin and ampicillin [81]. Aquatic photosynthetic organisms can also be affected.

Antiepileptics

Antiepileptic drugs act in the central nervous system (CNS) by reducing the overall neuronal activity. This can be achieved either by blocking voltage-dependent sodium channels (e.g. carbamazepine) or by enhancement of the inhibitory effects of the -aminobutyric acid (GABA) neurotransmitter (e.g. benzodiazepines) [82]. Carbamazepine is carcinogenic to rats but does not have mutagenic properties in mammals [83]. Moreover, this drug is lethal to zebrafish at the 43g/ L level and produces sub-lethal changes in *Daphnia* sp. at 92g/ L [83]. Regarding aquatic organisms, it can be deduced that carbamazepine does have harmful proclivity since most of the acute toxicity data were harvested from trial concentrations between 10 and 100mg/ L [84].

Beta-blockers

Beta-blockers act by competitive inhibition of beta-adrenergic receptors, a class of receptors critical for normal functioning in the sympathetic branch of the vertebrate autonomic nervous system in vertebrates. Within the most commonly used beta-blockers propranolol is a non-specific antagonist, blocking both 1 and 2-receptors while metoprolol and atenolol present 1-receptors specificity [85]. Fish, like other vertebrates, possess β -receptors in the heart, liver and reproductive system [86,87] so that prolonged exposure to drugs belonging to this therapeutic class may cause deleterious effects. From a two weeks study, it was observed that exposure to 500g/ L of propranolol reduced growth rates of Japanese medaka [88]. Plasma steroid levels were altered in both male and female fish even at concentrations as low as 1 g/ L propranolol. Exposure to concentrations of 0.5 and 1g/ L resulted in a

decreased egg production. On the other hand, acute exposure of rainbow trout to 70.9g/ L of propranolol showed no significant reduction in its heart rate [89].

Antineoplasics

Antineoplastic drugs are designed to kill cells that are proliferating excessively such as those found in pathological cancer conditions. Therefore, a similar effect on any other growing eukaryotic organisms is expected [89]. Pharmaceuticals belonging to this therapeutic class possess genotoxic, mutagenic, carcinogenic, teratogenic and fetotoxic properties and can constitute (in their native form) from 14 to 53% of the administered drug excreted in urine. Cyclophosphamide and ifosfamide ecotoxicity predicted by ECOSAR have yielded EC_{50} values of 8.2 and 70 mg/ L for algae and fish respectively, whereas the freshwater flea *D. magna* registered a LC_{50} of 1795 mg/ L [90]. The antineoplastic drug cyclophosphamide has been detected in hospital effluents at concentrations ranging from 19 ng/ L to 4.5g/ L [91], in STP influents [92,93] and effluents [92,93] and in surface waters [18]. Other antineoplastic pharmaceuticals detected to date have been in the order of ng/ L. However, as chronic toxicity data is very sparse, further studies are required to elucidate the potential effect of life-cycle exposure to these compounds in aquatic organisms.

X-ray contrast media

Contrast media are used as diagnostic tools for capturing detailed X-ray images of soft tissues. Iodinated X-ray contrast media are highly hydrophilic substances that are widely used and eliminated almost non-metabolised. STP removal processes are usually ineffective and for this reason they persist for a long time in the environment. As X-ray contrast media do not show biological activity, their presence might not represent a threat to public health [84,94]. Toxicity tests have shown that iopromide or its main metabolite do not have a toxic effect in luminescent bacteria, algae (*Scenedesmus subspicatus*), daphnids or fish (*D. rerio*, *Leuciscus idus*) even at concentrations as high as 1g/ L [94]. Contamination by X-ray contrast media has been reported in different aquatic environments. Media have been detected in STP influents and effluents, surface waters, groundwaters and even drinking water at concentrations that can reach few g/L [91,93,94]. Although accepting that X-ray contrast media do not exhibit toxic effects at high concentration levels, additional studies should be undertaken with a view to evaluating chronic effects, due to continuous exposure of aquatic organisms to these pharmaceuticals.

Conclusions and Futuristic Approach

Today, the presence of pharmaceuticals in the environment is being reported worldwide. Furthermore, new data on the sources, fate and effects of pharmaceuticals in the environment, seems to indicate the possibility of a negative impact on different ecosystems and imply a threat to public health. For this assumption, data from acute and chronic ecotoxicity tests on species belonging to different trophic levels such as bacteria, algae, crustaceans and fish among others, is relevant to illustrate the several adverse effects that environmental exposure to measured concentrations of these contaminants can

have. On literature, the principal toxicological endpoints/studies that are described are growth, survival, reproduction and immobilization of species, comparatively to transgenerational and population level studies that are still sparse. This demonstrates the lack of data relatively to long-term exposure of non-target organisms and principally how a continuous exposure, during several generations, may affect a whole population. To our knowledge, just one work followed the impact of a pharmaceutical in a fish population throughout seven years, showing how ethinylestradiol negatively affect the fish population, leaving them near of the extinction. In the near future, the evaluation of chronic toxicity effects should be set out as a priority for the scientific community since simultaneous exposure to pharmaceuticals, metabolites and transformation products of several therapeutic classes are unknown and whose probable effects on subsequent generations should be assumed. Another example of missing data is what occurs with statins. Nowadays, they are the blood lipid lowering agents most used all over the world, although toxicity data relatively to them is almost non-existent and limited to the active substances simvastatin and atorvastatin. It is also important to assess the presence of pharmaceuticals and/or their metabolites and transformation products in several environmental compartments in different countries with a view to gaining reliable knowledge of the contamination levels. Only with further available information will be easier to improve existing legislation in order to protect humans, animals and ecosystems from the threat posed by the presence of pharmaceuticals in the environment.

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